S. aureus- A review on the pathogenesis and molecular profiling by RAPD markers

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ABSTRACT

ESKAPE is a group of pathogens which constitute a highly concerning group of drug resistant bacteria, which are reported to cause nosocomial infections throughout the world. This is a basic review comprises a general introduction of ESKAPE pathogens namely: *Enterococcus faecium*, S*taphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, Enterobacter species along with the clinical threats and mechanism of their antibiotic resistance. The review specifically focuses on *Staphylococcus aureus* as a pathogenic organism, including its epidemiology across the world and in India, its pathogenesis, carriers, virulence factors along with the prevention and control measures. Further, the molecular characterization techniques have been included with main focus on use of Random Amplified Polymorphic DNA (RAPD) technique for characterization of S. aureus strains enlisting the primers used in the RAPD profiling conducted by various research groups along with the conclusion drawn from these experiments.

Keywords - *Staphylococcus aureus*, RAPD-PCR, characterization, antibiotic resistance, prevalence, pathogenesis.

I. Introduction

ESKAPE is the high concerning group of drug-resistant bacteria which includes both Gram-positive and Gram-negative species. These organisms are the leading cause of nosocomial infections throughout the world, ranging from wound infections and ventilator associated pneumonia to sepsis. They show multidrug resistance due to their ability to escape the biocidal action of antimicrobial drugs by representing new patterns in pathogenesis, transmission and resistance and this is one of the biggest challenges in clinical practice. Drug resistance is among the top three threats to public health in the world and is often caused by excessive drug usage or prescription, improper use of antimicrobials, substandard pharmaceuticals and continuous evolution among strains. Hence, ESKAPE pathogens are associated with a high risk of mortality and increased economic costs. However, increasing understanding of the virulence, resistance, transmission and pathogenicity in these organisms can lead to new strategies for developing new antimicrobial options.

ESKAPE comprises of following pathogen:

1.1 Enterococcus faecium (E)*:*

E. faecium is a Gram-positive, facultative anaerobe, opportunistic pathogen, often involved in HAIs (Hospital acquired infections), especially affecting immunocompromised patients [27]. It is coccus shaped, and can occur in pairs or chains. Its natural habitat includes gastrointestinal tract, oral cavity, and vaginal tract of a wide variety of animals. Enterococci typically causes Urinary tract infections, bacteremia, endocarditis etc. Increased levels of vancomycin resistance to enterococcal infections have been reported worldwide. *E. faecalis* and *E. faecium* are the third- to fourth-most prevalent nosocomial pathogen worldwide [21].

1.2 Staphylococcus aureus (S):

Staphylococcus aureus is a Gram-positive, cocci and often occurs in clusters resembling a bunch of grapes and is a causative agent of a wide range of infectious diseases such as skin infections, bacteraemia, endocarditis, pneumonia etc. The organism is well-known for its ability to show resistance or withstand to various antibiotic classes. The emergence and spread of the methicillin-resistant strain of *S. aureus*, called methicillin-resistant S. aureus (MRSA) has led to high morbidity, high mortality and increased medical costs. Vancomycin has been a gold standard drug for many years but the emergence of resistance has hampered its clinical use [11].

1.3 Klebsiella pneumoniae (K):

K. pneumoniae is a prominent member of the Gram-negative Enterobacteriacae family along with *Escherichia coli*, and together they lead to an increasing pathological threat [27]**.** It is an encapsulated, non-motile, facultative anaerobe bacillus. Virulence of an organism is shown by its capsule. Till date 77 different capsular types have been studied. Organisms grow on human mucosal surfaces such as oropharynx and GI tract. It causes pneumonia, blood infections, liver abscesses etc. It produces beta-lactamase which hydrolyse beta-lactam ring present in antibiotics. ESBL (Extended-spectrum beta-lactamase) shows resistance to third generation cephalosporin, carbapenem [4].

***1.4 Acinetobacter baumannii* (A):** *Acinetobacter baumannii* is a Gram-negative, bacillus, aerobic, pleomorphic and non-motile, opportunistic pathogen, can withstand dry conditions and shows environmental persistence. *A. baumannii* affect majorly immunocompromised individuals. It causes infection in lungs, blood, GI tract etc. It shows extensive resistance to first line antibiotics. The resistance to antibiotics is due to the presence of outer membranes.

1.5 Pseudomonas aeruginosa (P):

Pseudomonas aeruginosa is a Gram-negative, bacillus, facultative anaerobe, opportunistic pathogen, affects immunocompromised individuals and shows nutritional versatility. It spreads through improper hygiene and shows infections in the bloodstream, urinary tract, surgical wound etc. It is often associated with CF (cystic fibrosis) and cancer patients or burns victims [27]. *Pseudomonas aeruginosa* displays resistance to a variety of antibiotics, including aminoglycosides, quinolones and β-lactams [13].

1.6 Enterobacter species (E):

Enterobacter species are Gram-negative, motile, bacilli. It shows infections in the bloodstream, urinary tract, respiratory tract. These species are responsible for serious nosocomial infections (*E. aerogenes* is the leading cause) [8]**.** It shows MDR through plasmid-encoded ESBLs and carbapenemases [27].

We focus in this chapter on carriage, pathogenesis, epidemiology, and antibiotic resistance of *Staphylococcus aureu*s, along with RAPD characterization of its strains.

II. Staphylococcus aureus

Staphylococcus is a genus of Gram positive, it is a non-spore-forming cocci of the family *Micrococcaceae* and is frequently found as a normal human microbiota of the skin and nasal cavity. Apart from being salt resistant, *Staphylococcus* is a facultative anaerobe. When

it is stained, it appears in small groups or clusters (staphylo = cluster). It is either beta-hemolytic or non hemolytic (called gamma hemolysis). *Staphylococci* which are pathogenic can produce a variety of virulence factors, which include toxins, coagulase, leukocidins, and hydrolytic enzymes that can damage the underlying tissues. There are five organisms to be considered as possible human pathogens in this genus: *S. aureus, S. epidermidis, S. saprophiticus, S. haemolyticus*, and *S. hominis* but the first three are the most distinguished. *S.aureus* is generally considered to be the most common of the three strains and is isolated from the other two in that it is the only one that can coagulate plasma [15].

Staphylococcus aureus is both a human commensal and a major human pathogen and it causes a number of infectious diseases both in hospital and community areas. It causes a wide range of infectious diseases, such as skin infections, bacteraemia, endocarditis, food poisoning etc., because of its ability to express number of virulence factors that aid in the establishment of infection thus making it easier to attach the tissues, and tissues invasion and evade the host's immune response. The bacteria colonize on skin and nasal passage. Ecological niche for this species is the human body. MRSA is known as the world's leading blood-borne pathogen (BSIs).

Anemic, weak individuals and people with metabolic disorders (e.g. diabetes etc.) were known to reduce their resistance to staphylococcal disease [2].

Reports from 2019, the Staphylococcus genus includes more than 50 strains proposed as well as official, which include both coagulase-positive staphylococci (CoPS) and coagulase-negative staphylococci (CoNS) [19].

2.1 Carriers

It is an infective organism capable of transmitting disease to another organism without showing the clinical signs of the disease. Carriage pattern are of 3 types and were distinguished over time:

1) Persistent carriers: Same type of strain is always carried by 20% of people.
2) Intermittent carriers: The vast majority of people (±60%) harbors *S. aureus* intermittently, and with varying frequency strain changes.
3) Non-carriers: The minority (±20%) approximately never carry *S. aureus* [18].

S. aureus nasal carriage among the population, in general, varies. Nasal carriage is more prevalent and found to occur over the years. High carriage rates were seen in *S. aureus* skin infection, insulin-dependent diabetes mellitus, hemodialysis, CAPD, intravenous drug addicts, and HIV patients [18]. Nasal carriage rates in adults show global variation, in China the prevalence of nasal *S. aureus* was 23.1%, in India it was 29.4%, 9.1% in Indonesia, 14.8% in Pakistan [28]. Prevalence of Throat and MRSA carriage observed in some populations.

Age is a determining factor in SAB cases, and high levels of infection occur even at extremes of life. A slight increase in incidence in old age. Male gender is often associated with an increase in SAB incidence. HIV and hemodialysis patients showed increased risk for SAB.

At birth the babies are easily colonized but nasal carriage drops dramatically in the first year of life. The prevalence of *S. aureus* in the nasopharynx in young children is quite

stable between 20% and 30% until it exceeds 40-50% and remains there from 6 to 12 years of age after which it decreases slightly to about 25% at age 18. Adult diabetic recipients show high levels of *S. aureus* carriage [28].

Based on the laboratory evidence and the inputs of clinicians, ID physicians and clinical microbiologists the drug resistant pathogens were classified into three groups. MRSA belongs to Group III which includes drug resistant bacteria that are responsible for only a small number of infections but detection and early prevention of such infections can have significant impact on public health and need to be carefully watched in future. Thus, continuous monitoring and prevention efforts are required [3].

III. Pathogenesis

Staphylococcus aureus causes various cutaneous and soft tissue infections in humans which includes impetigo, cellulitis and infected ulcers and wounds. It also causes life-threatening infections, such as bacteremia, abscesses, meningitis, endocarditis and sepsis [22]. Pathogenesis of *S. aureus* is described in Figure 1, which highlights the anatomy of cutaneous infection.

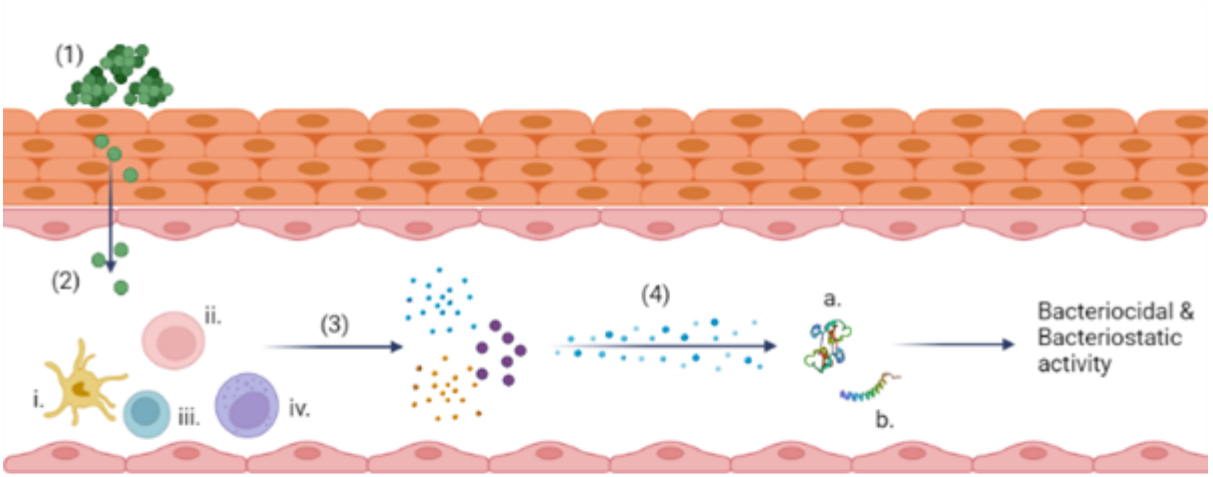


Figure I. Anatomy of cutaneous infection: (1) *S. aureus* infection; (2) Invasion to host and rupturing of epidermal barrier; (3) Release of proinflammatory cytokine, chemokine and adhesion molecule by the resident immune cells of skin such as – i. Langerhans cell, ii. T cells, iii. B cell, iv. NK cell; (4) The proinflammatory cytokine produces a. β-defensins and b. cathelicidins which are antimicrobial peptide and shows bacteriostatic and bactericidal activity [22]

S. aureus expresses many virulence factors which help them in attachment to host cells, destruction of the host's immune shield, invasion in tissue, causing sepsis and show toxin-mediated syndromes [11]

Table 1: Different virulence factors which help *S. aureus* by attaching to host tissues, breaking/evading the host immunity, tissue invasion and induce toxinosis along with their characteristics [11].

Factors	Characteristics

Helping attachment to host tissues	
Microbial Surface Components Recognizing adhesive matrix molecules (MSCRAMM)	Cell surface proteins which interact with host molecules such as collagen, fibronectin & fibrinogen, thus, facilitate the tissue attachment. Staphylococcal protein A, fibronectin-binding proteins A and B, collagen-binding protein & clumping factor A & B belong to this family. They are also involved in host immune evasion
Breaking/evading the host immunity	
Protein A	It binds to Fc portion of immunoglobulin, prevents opsonization, functions as superantigen & limits the host immune response
Alpha-toxin (Alpha hemolysin)	It was the first bacterial exotoxin to be identified as a cell membrane pore former which causes cell leakage & death
Tissue invasion	
Proteases, lipases, nucleases, hyaluronatelyase, phospholipase C, metalloproteases (elastase), & Staphylokinase	These extracellular enzymes cause tissue destruction and, thereby, help in bacterial penetration into tissues.
Induces toxinosis	
Enterotoxins	*S. aureus* produces a battery of enterotoxins which are potent gastrointestinal exotoxins. The Staphylococcal food poisoning is an intoxication which results from consumption of foods containing sufficient amount of preformed enterotoxins
Toxic shock syndrome toxin -1 (TSST-1)	TSST-1 & some of enterotoxins are called pyrogenic toxin superantigens. TSST-1 causes toxic shock syndrome especially in menstrual women
Exfoliative toxins A and B	Serine proteases which selectively recognize and hydrolyze desmosomal proteins in the skin. ETs cause staphylococcal-scalded skin syndrome, a disease predominantly affecting infants

Table 2: in vitro data on proteins responsible in various pathogenic functions [29]

Protein	Target	Function
1. Complement inactivation		
Aureolysin	C3	C3 Interfere in the deposition of complement on its surface
Ecb	Complement inhibition C3d	Complement inhibition
Efb	C3d and fibrinogen	C3d and fibrinogen Bind to C3d and inhibit C3bBb and C5 convertase binds fibrinogen and prevents fibrinogen interaction with αMβ2, an integrin on neutrophils that activates pro-inflammatory responses, as well as fibrinogen-mediated platelet activation
SSL7*	IgA and C5 (complement protein)	binds to human IgA and complement C5, interfering with IgA binding to FcαRI, the production of C5a and the oxidative burst of phagocytes result in Phagocytosis and complement inactivation
Staphylococcal complement inhibitor (SCIN)	C3 convertase (C3Bb)	Associates with C3 convertase and prevents the production of C3a, C3b and C5a, result in hindering the complement activation
2. Chemotaxis inhibition		
SSL11	PSGL1	interfere with the binding of neutrophils to P-selectin result in inhibition of neutrophil movement
Staphyopain (cysteine protease)	CXCR2 (chemokine receptor)	Cleave CXCR2 N-terminal and make it unresponsive

SSL5	PSGL1, GPCRs, GPIbα and GPVI Chemotaxis and platelet inhibition	Bind to PSGL1 and interfere its binding to P selectin results in inhibition of neutrophil movement*, it binds to the glycosylated amino termini of G protein-coupled receptors result in interfering with chemokine- and anaphylatoxin mediated activation of neutrophils and at last it shows platelet inhibition
FLIPrL	FPR1 and FPR2	Inhibit receptor signaling function
CHIPS	FPR1 and C5aR	Bind to its target and stops the movement of neutrophil and monocyte
3. Phagocytosis inhibition		
SSL10*	IgG1 (human and non-human primate)	inhibits IgG1 binding to Fcγ receptors and the phagocytosis of IgG1-opsonized bacteria by immune cells
Capsule	Not known	protects Staphylococci from neutrophil phagocytosis through opsonins
4. PMN Lysis		
PVL	C5aR on neutrophils, monocytes and macrophages	PVL not only exerts its lytic activity on target host cells but can also facilitate the priming of human polymorphonuclear leukocytes by pro-inflammatory stimuli
LukED	CCR5, CXCR1 and CXCR2	LukED binds to chemokine receptors and triggers lysis of macrophages, dendritic cells, T cells,PMN
5. Bacterial replication		
PSMα3N22Y (Allelic variant of PSM present in CC30 strains MRSA lineage)	Unlike PSM (Phenol-soluble modulins) which binds to FPR2 (formyl-peptide receptor 2) to reduce cytotoxicity, it shows diminished FPR2 signaling	Enhance bacterial replication and establishment of Abscess lesion in renal tissue

IV. Prevalence & Epidemiology

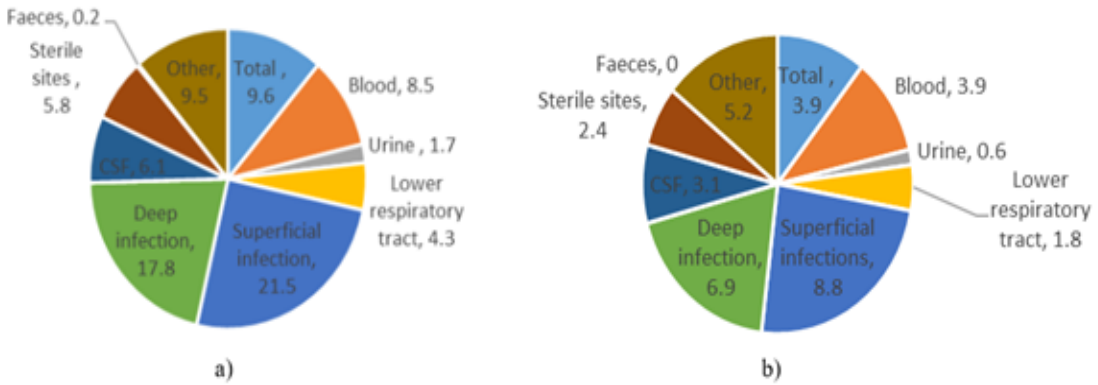
The overall proportion of *S. aureus* and MRSA in 2020 across India was 9.6% and 41.4% respectively. There were significant differences in MRSA rates observed between the various zones of India. The highest in the North (54.1%), followed by east (48.9%), west (39.3%) and southern zone (33.4%), with RC04 having the lowest rate of 26.8%. The VISA and hVISA prevalence in the country was 2.3% and 4% respectively.

Trend analysis over the years 2016 – 2020 show a steady decline in the isolation rates of *Staphylococcus aureus* from 13% to 9.6% in 2016 to 2020 respectively whereas, the rates of MRSA initially decreased form 2016-2017 then increased to 41.4% in 2020 as shown in figure 4. Among the different specimens, the highest rates of isolation of *S. aureus*, MRSA and MSSA was from Superficial infection (21.5%, 8.8% and 12.6% respectively) as indicated in figure 3 and Table 3. Among clinical settings, *S. aureus*, MRSA and MSSA were predominantly isolated from OPD with rates of 13.8%, 5% and 8.7% respectively. These trends are indicated in Table 4 [3]. Prevalence of MRSA has been studied worldwide. Figure 4 depicts the resistance of *S. aureus* to oxacillin.

Table-3: Trends of Isolation of *S. aureus*, MRSA, MSSA from various specimens in 2020

	Total	Blood	Urine	LRT	SI	DI	CSF	SS	Feces	Other
S. aureus	9.6	8.5	1.7	4.3	21.5	17.8	6.1	5.8	0.2	9.5
MRSA	3.9	3.9	0.6	1.8	8.9	6.9	3.1	2.4	0	5.2
MSSA	5.6	4.6	1.1	2.5	12.6	10.5	3	3.4	0.2	6.1

Abbreviations: LRT - Lower Respiratory Tract; SI - Superficial Infection; DI - Deep Infection; CSF - Cerebrospinal Fluid; SS - Sterile Sites. Source: AMR surveillance Network, Indian Council of Medical Research, 2020 [3]



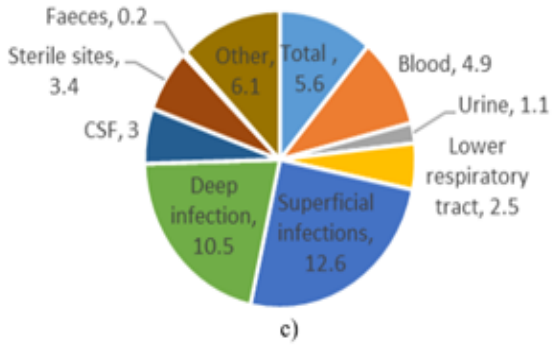


Figure-3: Isolation trends of *S. aureus*, MRSA and MSSA from blood, urine, lower respiratory tract, superficial infections, deep infections, Cerebral spinal fluid, sterile sites and faeces in 2020. The numbers represent the percentage of the above microbes isolated from various sources. a) Trends of isolation of *S. aureus* from various specimens in 2020; b) Trends of isolation of MRSA from various specimens in 2020; c) Trends of isolation of MSSA from various specimens in 2020.

Source: AMR surveillance Network, Indian Council of Medical Research, 2020 [3]

Table-4: Distribution of *S. aureus*, MRSA, MSSA from all specimens across OPD, ward and ICU in 2020

	Total	OPD	Ward	ICU
S. aureus	9.6	13.8	9.5	4.8
MRSA	3.9	5	4.1	2.3
MSSA	5.6	8.7	5.3	2.4

Source: AMR surveillance Network, Indian Council of Medical Research, 2020 [3].

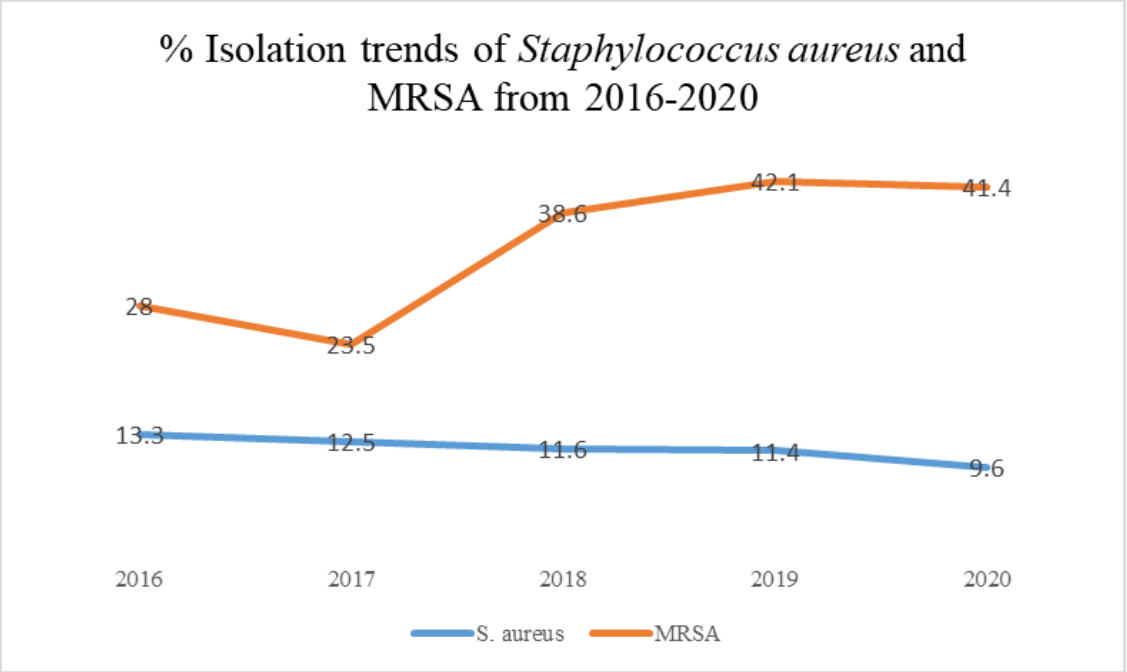


Figure-4: Yearly isolation trends of *Staphylococcus aureus* and MRSA from 2016-2020. The trends show decline in isolation of *S. aureus* while increase in isolation of MRSA strains from 2017-2020. The percentage of isolation is mentioned in this figure. Source: AMR surveillance Network, Indian Council of Medical Research, 2020 [3]

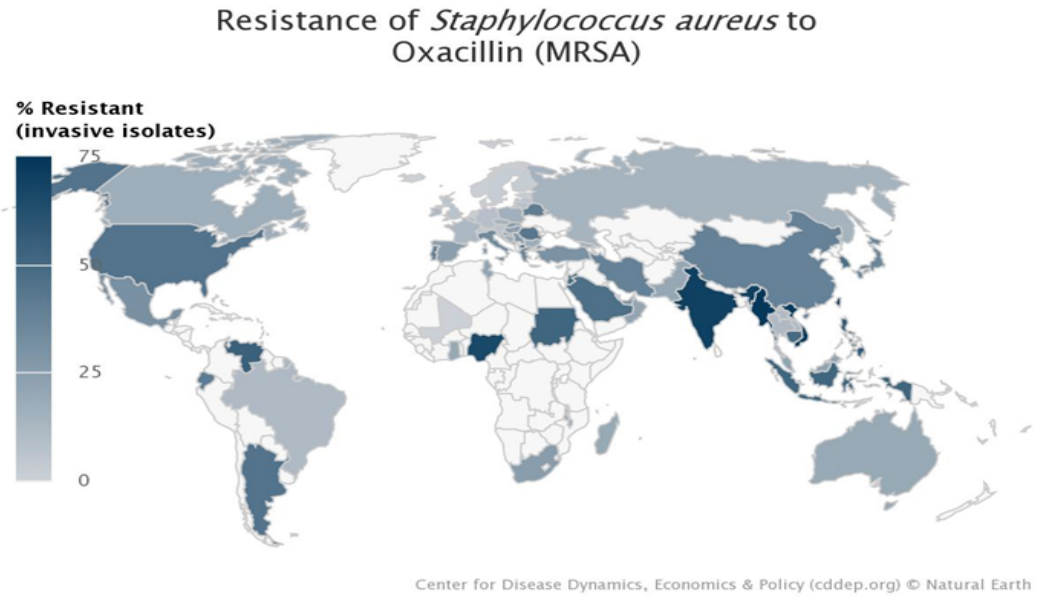


Figure-5: Resistance pattern of MRSA worldwide. The resistance map shows the percentage of resistance isolates. The percentage in different countries is as follows: USA(45%, 2016); Canada (17%, 2014); Mexico (31%, 2015); Brazil (11%, 2018); Argentina (45%, 2018); Nigeria(66%, 2018); India (70%, 2018); China (38%, 2017); Russia (14%, 2018); Spain (24%, 2018) Vietnam (73%, 2016); Australia (19%, 2017), Indonesia (52%, 2018) Source: CDDEP [30]

v. Characterization Technique Of *S. Aureus*

S.aureus infections continue to increase rapidly, and with an awareness of their constantly changing epidemiology comes the need for quick and trustworthy methods for the characterization of isolates. Nowadays, most classification schemes are based on molecular methods rather than phenotypic methods, providing more discriminatory power.

There are 4 methods available which are frequently used in identification of bacterial isolates:

- Morphological identification by Gram staining
- Biochemical test
- Molecular identification
- Antibiotic susceptibility

5.1 Morphological identification-

The presence of staphylococci might first be suspected after examination of a direct Gram stain. The organism is isolated by streaking from the clinical specimen (or from a blood culture) onto solid media such as blood agar, tryptic soy agar or Brain heart infusion agar. Specimens that may be contaminated with other microorganisms can be seeded on mannitol salt agar medium containing 7.5% sodium chloride. This allows the growth of halo-tolerant staphylococci. Identification of *Staphylococcus aureus* was achieved by fermentation of mannitol on selective agar (medium yellow coloration), positive reaction in the coagulase test, and microscope observation of Gram-positive cocci in clusters from a direct extended Gram stain.

5.2 Biochemical Test-

It detects the biochemical reactions which are occurring in the cell such as catalase test, coagulase test, mannitol fermentation test. Some of the tests are given in Table 6 and Table 7.

Table-6: Types of biochemical tests which are used in biochemical identification of *S.aureus*.

BASIC CHARACTERISTIC	PROPERTIES
Capsule	Non-capsulated
Catalase	Positive
Citrate	Positive
Coagulase	Positive
MR (Methyl Red)	Positive

OF (Oxidative Fermentative)	Positive
Spore	Non-sporing
Urease	Positive
VP (Voges Proskauer)	Positive

Table-7: Fermentation of different types of sugar for the identification of *S.aureus*

Fermentation of sugar	**Result**
Arabinose	Negative
Cellobiose	Negative
DNase	Positive
Fructose	Positive
Galactose	Positive
Glucose	Positive
Lactose	Positive
Maltose	Positive
Mannitol	Positive
Mannose	Positive

Ribose	Positive
Sucrose	Positive
Trehalose	Positive
Xylose	Negative

5.3 Molecular characterization-

Different molecular techniques can be used for characterization such as plasmid analysis, Chromosomal DNA Restriction Endonuclease Analysis, Southern blot analysis of RFLP, PCR based on typing methods (RAPD, RFLP) DNA sequence analysis based typing methods, SCCmec typing and toxin gene profiling typing. Table-8 describes these methods of molecular characterization.

Table-8:Different types of molecular techniques which are used in characterization of *S.aureus* [23]

Molecular Technique	Description
Plasmid analysis	It differentiates isolates according to the variety and length of plasmids which can be then measured with the aid of electrophoresis. It is a easy and simple method.
Chromosomal DNA Restriction Endonuclease Analysis (REA)	Restriction endonucleases cleave DNA on specific nucleotide recognition sequences. These fragments are separated according to size by agarose gel electrophoresis.
Southern blot analysis of RFLP	Restriction fragments which are generated are transferred on to nitrocellulose membranes. The fragments containing specific sequences are then detected by labeled DNA probes.
Pulsed field gel electrophoresis	It is a variant of conventional agarose gel electrophoresis in which the orientation of the electric field throughout the gel is changed periodically. This alteration allows large fragments to be separated according to size, minimizing the overlapping of fragments. The restriction enzyme Sma I is widely used in the study of MRSA strains by PFGE.

Polymerase chain reaction (PCR)- based typing methods	PCR techniques for identification of complex DNA molecules offer fast methods for the discrimination of MRSA strains.Among the various PCR based typing techniques,polymerase chain reaction or random amplified polymorphic DNA (AP-PCR/RAPD) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) have been reported as more useful for typing of MRSA strains.
RAPD	This involves the random amplification of a target DNA segment using small primers of random nucleotide sequences of unknown homology to the target sequence. The number and size of the fragments generated during PCR are the basis for specifying the isolate type . This technique does not require digestion of the amplified fragment. It is simple and fast. To be discussed later in this review.
PCR-RFLP	PCR-RFLP is based on the creation of restriction fragment length polymorphisms by amplifying a specific DNA fragment and then digesting the amplified product with restriction enzymes. These polymorphisms allow strains to be distinguished. The coagulase (coa) gene RFLP and the spa gene RFLP are widely used to differentiate MRSA strains.
DNA sequence analysis-based typing methods	DNA sequencing is an objective method of genotyping because the genetic code can be easily ported, stored, and analyzed in relational databases. Two different strategies were used to type isolates by analyzing DNA sequences: multiple locus sequence typing (MLST) and single locus sequence typing (SLST).
Multilocus sequence typing (MLST)	MLST has been reported to be useful for clonal evolution studies of MRSA. It is a genotypic descendant of MLEE based on sequencing of several housekeeping genes. Seven housekeeping genes studied by various researchers are arcC, aroE, glpF, gmk, pta, tpi, and yqiL. The different sequences of each housekeeping gene are assigned separate alleles, and each MRSA strain is determined by the alleles of seven genes. MLST was used in conjunction with PCR analysis of the staphylococcal cassette chromosomal element (SCC mec) mec to determine the clonal type of the MRSA strain.
Single-locus sequence typing	Single locus sequencing (SLST) is used to compare sequence variations in a single target gene. The genes selected are usually short sequence repeat (SSR) regions that are polymorphic enough to provide useful resolution. Protein A (spar) and coagulase (coa) genes of the MRSA strain with 24 bp tandem repeats. and 81 bp have been extensively studied and reported that MRSA strains can be distinguished by determining repeat sequence numbers in the spa X region of the gene.

Staphylococcal cassette chromosome mec (SCCmec) typing	S. aureus acquires resistance to methicillin due to a mobile genetic element of the staphylococcal cassette chromosome mec (SCCmec) containing the mec A gene complex and the ccr gene complex. Several mec and ccr allotypes were found in the SCCmec element. Currently, there are eight main types (I through VIII) of SCCmec, and many subtypes of MRSA strains. Each type of SCCmec exhibits resistance to different antibiotics. Differences in these types of SCCmec formed the basis for the differentiation of MRSA strains.
Toxin gene profile typing	MRSA strain produces a variety of toxins, including toxic shock syndrome toxin (tsst1), enterotoxin, and exfoliating toxin. The gene encoding the enterotoxin is located on the pathogenic island of Staphylococcus aureus. Other toxin genes, such as the Panton Valentine leukocidin (PVL) gene, are carried by bacteriophages and are easily transferred between lineages. Therefore, the strain toxin gene profile can be used as an important epidemiologic marker for typing MRSA strains.

5.4 Antibiotic Susceptibility Test-

The susceptibility of the *Staphylococcus aureus* isolates to various antimicrobial agents was determined by disc diffusion method. This method is based on inhibition of bacterial growth which is measured under standard conditions. In this test, a culture medium (Mueller-Hinton agar), is uniformly and aseptically inoculated with the test organism and a filter paper disc impregnated with a specific antibiotic at a specific concentration is placed on the medium. The organism grows on the agar plate while the antibiotic "acts" to inhibit growth. If the organism is sensitive to a certain antibiotic, no growth will occur around the disc containing the antibiotic. Therefore, an "inhibition zone" can be observed and measured to determine the susceptibility to an antibiotic for that particular organism. The measurements are compared to the standards established by the Clinical and Laboratory Standards Institute (CLSI). Based on the criteria, organisms can be classified as being resistant (R), intermediate (I) or susceptible (S).

VI. Antibiotic Resistance

Staphylococcus aureus also shows Multidrug resistance (MDR) leading to evolution of antibiotic resistance strains. MDR in *S. aureus* is classified into primary MDR, secondary MDR, and clinical MDR [25].

Initially, penicillin was used against the infections of *S. aureus*, but in a few years the microbe became resistant to the antibiotic via β-lactamase enzyme. Later, methicillin was used but it led to evolution of Methicillin resistant *Staphylococcus aureus* (MRSA) strains as, Methicillin-susceptible *Staphylococcus aureus* (MSSA) strains started adopting *mecA* gene. MRSA strains are further classified as hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA). Vancomycin was used as an antibiotic drug against MRSA strains but after a few years, *Staphylococcus aureus* also became resistant to vancomycin antibiotics. The *S.* aureus strains resistant to vancomycin were classified as – vancomycin-resistant *Staphylococcus aureus* (VRSA), vancomycin-intermediate *Staphylococcus aureus* (VISA) and heterologous vancomycin-resistant *Staphylococcus aureus* (hetero VRSA) strains [25], [12]. The resistance gene and its product, transmission and resistance mechanism has been discussed in table-9.

Various mechanisms of antibiotic resistance by *Staphylococcus aureus* have been shown in Figure-6. Different genes and their mechanisms of antibiotic resistance have been well discussed by Otarigho & Falade [26]**.** They also discussed that one gene can have more than one antibiotic mechanism. Further Foster complements by describing in detail the antibiotics to which *S. aureus* exhibit resistance.

Table-9: The resistance genes of penicillin, methicillin, and vancomycin antibiotics and its product, transmission and resistance mechanism [10]

S. no.	Antibiotics	Resistance gene	Gene product	Gene Transmission	Mechanism of resistance
1.	Penicillin	*blaZ*	β-lactamase	The gene is carried by transposon Tn552 or Tn552-like elements located on large plasmids integrated into chromosome.	Hydrolytic degradation of penicillin.
2.	Methicillin	*mecA*	PBP2a	The gene is a part of the genetic element called Staphylococcus cassette chromosome (SCC *mec*). It is transferred to Methicillin susceptible *S. aureus* (MSSA) strain via horizontal gene transfer.	The active site of serine transglycolylase-transpeptidase (TP) PBP2a lies in deep pockets, which are not accessible to β-lactam antibiotics.
3.	Vancomycin	vanA		A possible transfer of vanA operon from vancomycin-resistant *E. faecalis*. The Enterococcal plasmid containing vanA also contains the sex pheromone produced by *S. aureus*, proposes to be a potential conjugal transfer	Increase in the thickness of the cell wall and alteration in its architecture.

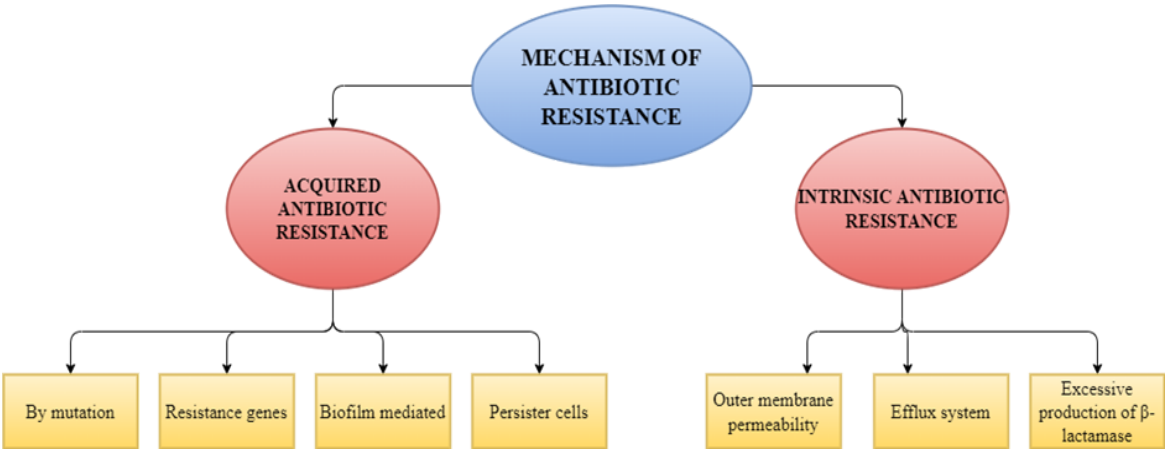


Figure-6: *Staphylococcus aureus* show two types of mechanisms of resistance – Acquired antibiotic resistance and Intrinsic antibiotic resistance. (By mutation: *S. aureus* acquires resistance to clindamycin and erythromycin via modification in ribosomal RNA methylase; By resistance gene: the bacteria acquire resistance gene by plasmid mediated transduction, transformation, and insertion of drug-resistant gene. Eg., *blaZ* gene.; Outer membrane permeability – *S. aureus* show resistance to aminoglycosides by decrease in membrane permeability and reduced drug intake.; Efflux drug systems – three types of multidrug protein pumps present in *S. aureus*: QacA, NorA, Smr. QacA is the most important drug in MRSA. AMR against tetracycline antibiotics occurs via Tet efflux pumps TetA(K) and TetA(L); Excessive production of β-lactamase leads to antibiotic resistance either via hydrolysis mechanism or pinching mechanism. [12]

VII. RAPD-PCR FOR CHARACTERIZATION OF *S. AUREUS*:

7.1 Results concluded from previous studies:

The RAPD-PCR technique has been adapted by various scientists to characterize the strains of *Staphylococcus aureus*. The isolates of *S. aureus* strains come from a variety of sources, including clinical samples from various hospitals, food, banknotes, and animal samples collected from various regions. Based on their experiments, the researchers have drawn some conclusions as given in table-10.

RAPD-PCR is a widespread genetic fingerprinting method. It has shown to act as a valuable tool in various applications as discussed further in this topic. RAPD of *S. aureus* is a user-friendly, faster technique, producing greater polymorphisms and requiring no radioactive materials. The method is helpful in assessing relationships between host origin, mutation and genetic variations among isolates of *S. aureus*. It could be used in tracing and controlling the sources and routes of transmission of *S. aureus* infections. The technique can be potentially used in tracking the spread of strains within hospitals and between the hospitals. It acts as a reliable and fast epidemiologic typing method for monitoring the spread of multidrug resistant MRSA strains via comparisons of DNA band patterns among microorganisms as these patterns vary with the mutation, virulence genes and host origin. It is a widely used technique for investigating the genetic variability of any nosocomial infections. Thus, it can be helpful in preventing the nosocomial infection caused by multidrug resistant MRSA strain, in epidemiological surveys, medical diagnosis, and in identification of new virulent strains and their origin [31] [5] [2] [7] [24].

Along with hospital acquired infection, RAPD-PCR can be successfully used to investigate the distributional and epidemiological relationship of CA-MRSA (Community associated MRSA) strains under careful reproducibility conditions and particular duplication of PCR runs, in order to obtain valid results [24].

TABLE-10: Conclusion of RAPD-PCR results obtained from samples collected from Banknotes, food, human infections, bovine milk sample, various hospitals, Clinical sample from various sources.

Source of Isolates	Key findings	Authors
Banknotes, Foods, Human infections, bovine mastitis milk	· Most polymorphism was seen in isolates of bovine mastitis whereas lowest polymorphism observed in isolates of human infections. No relationship between RAPD patterns and sources of isolates, with some exceptions. · 100% RAPD profile similarity level, i.e., genetically indistinguishable isolates. Some isolates from human infections were non typable, may be due to absence of specific sites for primer to bind. · Wide genotypic diversity of *S. aureus* strains confirmed. Use of a high number of primers for assessing the genetic relationship between *S. aureus*, increased the number of different strains.	Zare et al, 2019
Human infections, Bovine milk sample, food samples	· RAPD- PCR was used to investigate dissemination and clonal relatedness of *S. aureus* strains isolated from human, bovine and food samples. · Results suggested the transmission of these strains among different sources. RAPD-PCR analysis revealed 47 distinct profiles among all studied isolates, indicating the genetic heterogeneity. · Use of AP-7 primer in RAPD-PCR was concluded suitable in molecular typing, phylogenetic analysis, and detection of polymorphism in different isolates of *S. aureus*. However, some samples were indistinguishable by the primer used. · Clustering of isolates is majorly dependent on the source but in some isolates, strains from different sources share the same amplification profiles. Thus, suggesting that *S. aureus* can be transmitted from food of animal origin to humans. · *S. aureus* strains are predominantly host specific as certain host specificity is seen between bovine and human isolates. · *S. aureus* nasal carriers are more susceptible to nosocomial infections than non-carrier individuals.	Alni et al, 2017
MRSA strains from different hospitals, Turkey	· Researchers used 10 random primers out of which three yielded discriminatory patterns. These are- S224, S232, S395. These primers were individually able to distinguish strains obtained from different hospitals. · Primers used provided an advantage for using RAPD-PCR for potentially determining the clonal dissemination of MRSA strain, via formation of dendrograms based on the results of the primer used. · Strains isolated from the same hospital were closely related and placed together in the same group. The strains isolated	Idil & Bilkay, 2014

	from two of the hospitals were found to be similar, thus indicating that they might have emerged from one clone. · Choice of primer is the most critical factor and use of many primers contribute to occurrence of different banding patterns. High discriminatory power can be accomplished by use of several primers. However, the primers are sometimes insufficient to distinguish genetic differences among the related and unrelated strains. Thus, there is no specific primer for discrimination.	
Clinical samples from wounds, skin, nails, and urinary tract infection from patients.	· The primers used showed polymorphisms among individuals and each primer gave different genetic profiles. · Phylogenetic relationship among the isolates was constructed via dendrograms and isolates were further classified into major groups on the basis of the primers used. · Researchers found that identification of genetic diversity in *S. aureus* depends on sources of isolates, different host cells and occurrence of mutants. Specific bands generated in the RAPD can be related to host origins, mutations and virulence genes in areas of medicine, population biology and epidemiology. · RAPD isolates revealed the efficacy of the selected primers in determination of the similarity or variations among all isolates.	Banoon & Kadhim et al, 2019
Community acquired MRSA strains from anterior nares of healthy children.	· Primer 1254 showed best RAPD patterns w.r.t. number, distribution, and intensity of bands. It was further used for typing analysis of all strains. · Researchers inferred RT1 and RT2 as major prevalent strains circulating in the community. · Researchers showed that optimization of PCR conditions is essential for reliability and reproducibility of polymorphic patterns. As in their experiments, it was crucial to optimize the amount of DNA in each RAPD assay in order to ensure that non-specific bands were absent. · According to previous studies, polymorphism generated by RAPD was said to be almost the same as that yielded by RFLP, but researchers concluded that RAPD is almost beyond comparison in terms of simplicity, fastness and low-cost condition with most other molecular typing methods. · Researchers from this study concluded that RAPD fingerprinting can classify isolates of MRSA into clusters through which the relationship of strains can be further evaluated.	Mobasherizadeh et al, 2016
Clinical samples from hospitals, Iraq	· RAPD results reveal the efficacy of selected primers i.e., OPB-10, OPX-01 in determination of the similarity and variations among all isolates.	Amalraqibshamran et al, 2018

	· The DNA band patterns help in identification of the strain of *S. aureus* such as Sa1 strain contain special bands amplified with most of the primers. · Researchers in their study noticed that the process of identification of genetic diversity of *S. aureus* depends on the host cells and sources of isolates. · RAPD markers discovered the relationship between genetic variation, mutations and host origin in *S. aureus* isolates.	
Clinical samples including urine, pus, sputum semen, ET secretion, and swabs from vagina, umbilical cord, nasal, throat, ear, eyes from different hospitals, Bangalore	· Construction of dendrogram for the banding profiles for each RAPD profile helps in rapid screening of multidrug resistant strains. This will also provide an opportunity for monitoring the emergence and spread of multidrug resistant MRSA strains between the locations. · Dendrograms for each primer showed that selected multidrug strains could be easily distinguished from each other. Different primers showed different similarity patterns between the strains analyzed. · The dendrograms indicated that the strains from two different locations within the city are placed in the different groups based on their genetic similarities. It also demonstrated that the distinct clustering of multi-drug resistant strains is due to intra-spread of MRSA strains between locations · The reason for this situation especially in developing countries might be due to antibiotic misuse, shortfalls in infection, control and urban migration which might increase the chance of spread of resistant strains in the healthcare and community. · Choice of primers is one of the most essential factors that helps in occurrence of different banding patterns. · Studies in Nigeria and Nepal demonstrated that urban residents are more likely to contain resistant bacteria than people residing in rural or provincial areas.	Debnath, 2015
Pus Samples from humans and animals such as cattle, dogs and buffaloes.	· In RAPD-PCR typing two-band patterns were similar in human and animal MRSA isolates, concluding that MRSA might have transferred from animal to human. Thus, proving that “the transfer of S. aureus between humans and cattle is possible” according to previous studies.	Jayshree et al, 2021

7.2 Majorly used primers

Primers are the short oligonucleotide sequences which initiate the replication of DNA. In RAPD-PCR experiments, the choice of primers is the most essential factor to be considered. Use of a large number of primers contributes to the occurrence of different banding patterns in order to distinguish the strains of *S. aureus* and assess the genetic relationship between them. They help to accomplish high discriminatory power [14].

Primers show polymorphisms among individuals and each primer gives different genetic profiles [5]. In an experiment researchers used 10 primers with the aim to identify appropriate primers to establish clonality of MRSA strains. They found that only three primers out of the ten primers used were individually able to distinguish the strains from various clinical samples. Thus, primers are sometimes unable to distinguish genetic differences among the related and unrelated strains. Also, there are no specific primers for discrimination between the strains [14].

Scientists have used some primers in the past that gave excellent RAPD patterns in previous experiments. The majorly used primers include - OPL-6, OLP-11, OLP-13 [31] [7] [24].

VIII. PREVENTION AND CONTROL

As *S. aureus* infection may be severe and may also become life-threatening. The preventive measures which can be taken which includes: 1. Overcrowding in places like schools, malls, military camps etc are susceptible to infection, so all are recommended to report minor or major infections, 2. Avoid contact with an infected person as well as contaminated items or surfaces, 3. Should follow proper hygiene.

To prevent surgical site infections (SSI) these standards should be adopted: 1) Preoperative screening and decolonization for *S. aureus*, including MRSA patients, 2) screening and decolonization of MRSA in healthcare workers.

Healthcare workers colonized with dangerous pathogens, including MRSA, are at high risk of spreading the infection, so they are avoided to be in contact with patients [17]. Prevention of transmission of CA-MRSA includes [6]:

1) By an individual: Regular hand hygiene to limit personal contamination and transmission an if skin lesion are present it is recommended to cover lesion with proper dressings or to consult from medical practitioner and sharing of personal items should be avoided that come in contact with their lesions
2) By an health care practitioners: Treatment of viral infections with antimicrobials should be avoided and patients are encouraged to take the complete course of prescribed antibiotics
3) By health authorities: Communication strategies to inform the general public and high-risk groups. Strategies for ensuring early diagnosis and appropriate treatment of skin infections and educate people regarding the proper use of antibiotic

Through recent studies and evidence, the use of mupirocin as prophylaxis for preventing infections of *S. aureus*, especially including carriers and surgical settings or patients receiving dialysis treatment. The combination of mupirocin and chlorhexidine is also effective against *S. aureus* infections. Chlorhexidine alone does not show any result. Currently, vaccines are not proven to be effective for *S. aureus* infection [9]. Some doctors recommend using the antibiotic mupirocin in the nostrils to remove staphylococci from the nostrils. However, abuse of mupirocin can lead to resistance to mupirocin, so this antibiotic is only used when people are more likely to get infected. For example, it is given to people who have not undergone certain surgery or who live in a household where skin infection is widespread. Some medical facilities regularly screen MRSA patients on admission. Some institutions screen only those who are at high risk of MRSA infection. B. Those who have to undergo specific surgery. During the screening, a sample taken from the nose is tested with a cotton swab. When MRSA strains are detected, humans are quarantined to prevent the spread of bacteria [20].

IX. CONCLUSION

ESKAPE, a group of drug resistant gram-positive and gram-negative bacteria, are the leading cause of nosocomial infections throughout the world. They escape the biocidal action of antimicrobial drugs by representing new patterns in pathogenesis, transmission and resistance. Drug resistance is the major threat to public health in the world caused by excessive and improper use of antimicrobials, substandard pharmaceuticals and continuous evolution among the strains, leading to high mortality and increased economic cost. Thus, new strategies have to be developed for developing new antimicrobial options.

There has been a major emphasis on characterization of strains of *Staphylococcus aureus* via RAPD in this chapter as well as its carriage, pathogenesis, epidemiology, and antimicrobial resistance.

Staphylococcus aureus, a gram - positive bacteria, is a causative agent of a wide range of diseases because of its ability to express a number of virulence factors which help in invasion and evasion in host

organisms. *S. aureus* shows three types of carriage patterns - persistent carriers, intermittent carriers and non carriers. The nasal carriage is more prevalent among *S. aureus* strains with a prevalence rate of 29.4% in India. Apart from it, age, gender, and immunocompromised patients are the major determining factors in SAB cases. Among the strains of *S. aureus*, MRSA belongs to group III of drug resistant bacteria according to ICMR annual report 2020.

S. aureus, causes various life threatening infections such as, bacteremia, abscesses, meningitis, endocarditis, and sepsis. Various cutaneous and soft tissue infections in humans are caused by *Staphylococcus aureus* which includes impetigo, cellulitis and infected ulcers and wounds.

When it invades to host by rupturing the epidermal barrier the host immune system get activated, first by release of proinflammatory cytokine, chemokine and adhesion molecule by the resident immune cells of skin (such as Langerhans cell, T cells, B cell, and NK cell); and then β-defensins and cathelicidins which are antimicrobial peptide produced by cytokine ultimately led to bacteriostatic and bactericidal activity.

To cause infection *S . aureus* have many virulence factors and proteins which help them invade, breaking or evading from host immune response to show toxic mediated syndromes.

The review highlights the decline in isolation rates of *S. aureus* over the years from 2016 - 2020, while an increase in rates of MRSA isolation from 2018 - 2020. Among the different specimens, highest rates of isolation of *S. aureus*, MRSA and MSSA was from superficial infections and lowest from feces. Among the clinical settings, in OPD, the highest rate of isolation of these species was observed. MRSA strains show a high resistance pattern worldwide. *S. aureus* has acquired resistance against various drugs over the years leading to development of antimicrobial resistance strains such as MRSA (methicillin resistant), VRSA (Vancomycin resistant), VISA, hetero VRSA. *S. aureus* shows acquired antibiotic resistance and/or intrinsic antibiotic resistance.

Due to changing epidemiology and increasing resistance rates, characterization of isolates becomes essential. There are four basic methods of characterization of *S. aureus* - i. Morphological techniques (based on Gram staining), Biochemical tests (for detection of biochemical reactions occurring in an organism. Through this technique, we can identify a given isolate, via observations of a set of biochemical reactions), molecular identification (it includes molecular techniques such as SSCmec typing, PCR based typing, Southern blot analysis, toxin gene profiling, etc.) and antibiotic susceptibility (it tests susceptibility and resistance of given organism towards different antibiotics).

The review mainly focuses on RAPD-PCR technique for characterization of *S. aureus* strains. This technique produces greater polymorphisms and is helpful in assessing relationships between host origin, mutation and genetic variations among isolates of *S. aureus*. Thus, it can be used in tracing and controlling the sources and routes of transmission of *S. aureus* infections and for tracking the spread of strains within hospitals and between the hospitals and monitoring the spread of multidrug resistant MRSA strains via comparisons of DNA band patterns among microorganisms as these patterns vary with the mutation, virulence genes and host origin. It can also be helpful in preventing the nosocomial infection caused by multidrug resistant MRSA strain, in epidemiological surveys, medical diagnosis, and in identification of new virulent strains and their origin**.**

Various conclusions drawn from RAPD - PCR results of *S. aureus* isolates include are - in RAPD PCR, optimization of PCR conditions and choice of primer is the most important factor and a high number of primers should be used for properly assessing the genetic relationships between *S aureus* strains. Certain sets of primers were concluded as more effective, however, there is no specific primer which can give reliable results and discriminate between the strains. The majorly used primers in various experiments include - OPL-6, OLP-11, OLP-13 Further, dendrograms can be constructed from banding patterns in order to establish relationships among the strains. *S. aureus* strains are predominantly host specific and the genetic diversity is due to source, host cells and occurrence of mutants. The banding pattern thus can be related to origins and mutations. Some strains of *S. aureus* can show transmission of from one host to another.

Thus, RAPD-PCR can act as a valuable tool for controlling *S. aureus* infections along with other prevention and control measures.

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